

Applicant: **Astra Pharma Inc.**
1004 Middlegate Road
Mississauga
Ontario L4Y 1M4
Canada

Title: **NOVEL COMPOUNDS WITH ANALGESIC
EFFECT**

Reference: **N 1571-1**

Inventors: **ROBERTS, Edward**
PELCMAN, Benjamin

NOVEL COMPOUNDS WITH ANALGESIC EFFECT

Field of the invention

5 The present invention is related to novel nitrogen ring compounds, to a process for their preparation, their use and pharmaceutical compositions comprising the novel compounds. The novel compounds are useful in therapy, and in particular for the treatment of pain.

Background and prior art

10

The δ receptor has been identified as having a role in many bodily functions such as circulatory and pain systems. Ligands for the δ receptor may therefore find potential use as analgesics, and/or as antihypertensive agents. Ligands for the δ receptor have also been shown to possess immunomodulatory activities.

15

The identification of at least three different populations of opioid receptors (μ , δ and κ) is now well established and all three are apparent in both central and peripheral nervous systems of many species including man. Analgesia has been observed in various animal models when one or more of these receptors has been activated.

20

With few exceptions, currently available selective opioid δ ligands are peptidic in nature and are unsuitable for administration by systemic routes. Some non-peptidic δ antagonists have been available for some time (see Takemori and Portoghese, 1992, Ann. Rev. Pharmacol. Tox., 32: 239-269. for review). These compounds, e.g. naltrindole, suffer from rather poor (i.e., < 10-fold) selectivity for the δ receptor vs. μ receptor binding and exhibit no analgesic activity, a fact which underscores the need for the development of highly selective non-peptidic δ ligands.

Thus, the problem underlying the present invention was to find new analgesics having improved analgesic effects, but also with an improved side-effect profile over current μ agonists and potential oral efficacy.

- 5 Analgesics that have been identified and are existing in the prior art have many disadvantages in that they suffer from poor pharmacokinetics and are not analgesic when administered by systemic routes. Also, it has been documented that preferred compounds, described within the prior art, show significant convulsive effects when administered systemically.

10

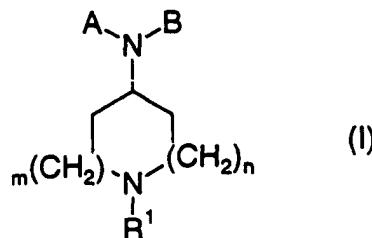
The problem mentioned above has been solved by developing novel compounds which possess a piperidine ring, which may be a 5-membered, a 6-membered or a 7-membered nitrogen ring, as will be described below.

15

Outline of the invention

The novel compounds according to the present invention are defined by the general formula (I)

20



wherein

m is 0 or 1;

25 n is 1 or 2;

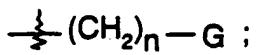
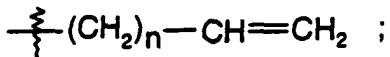
R^1 is selected from

hydrogen;

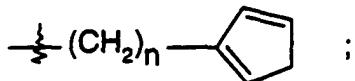
a branched or straight C₁-C₆ alkyl;

C₃-C₈ cycloalkyl;

5 C₄-C₈(alkyl-cycloalkyl) wherein alkyl is C₁-C₂ alkyl and cycloalkyl is C₃-C₆ cycloalkyl; benzyl;



where G is a hydroaromatic or a heteroaromatic group having 5 or 6 atoms, and where the
10 heteroatoms are selected from O, S and N; and



and wherein n = 0 or 1;

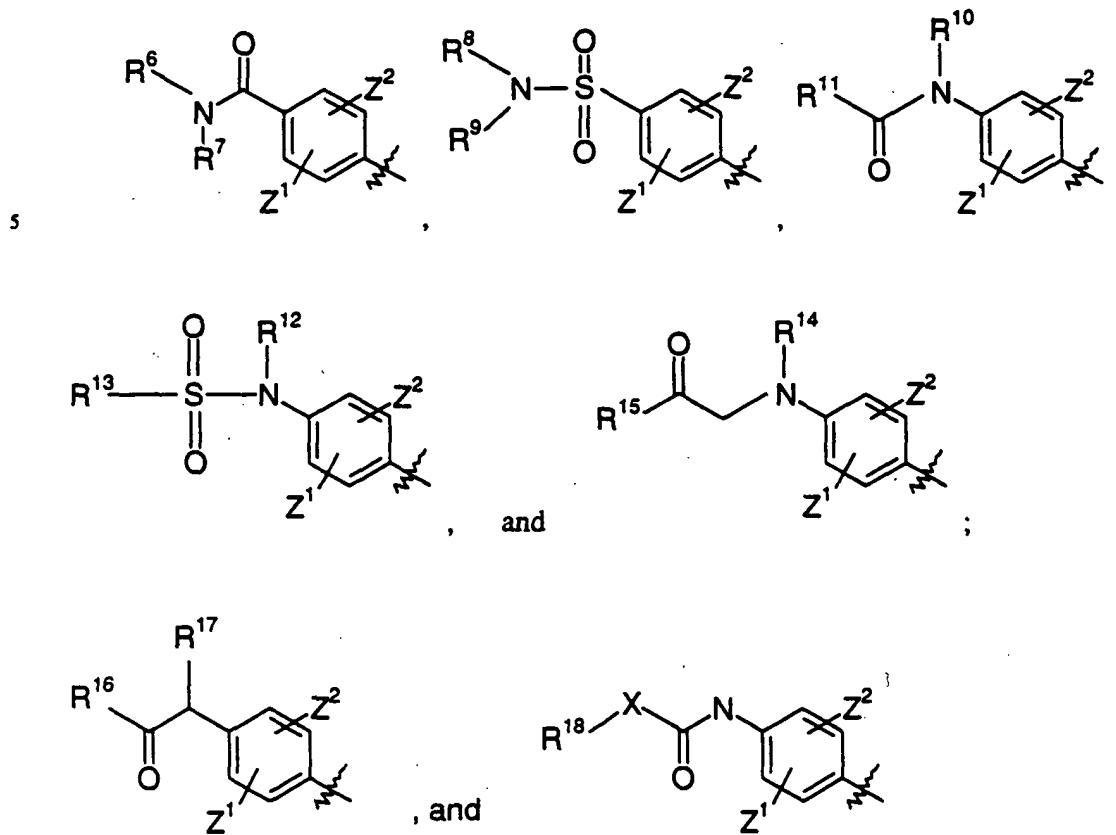
C₆-C₁₀ aryl; or heteroaryl having from 5 to 10 atoms selected from any of C, S, N and O;
15 wherein the aryl and heteroaryl may optionally and independently be substituted by 1 or 2 substituents independently selected from any of hydrogen, CH₃, (CH₂)_pCF₃, halogen, CONR⁵R⁴, COOR⁵, COR⁵, (CH₂)_pNR⁵R⁴, (CH₂)_pCH₃(CH₂)_pSOR⁵R⁴, (CH₂)_pSO₂R⁵, and (CH₂)_pSO₂NR⁵, wherein R⁴ and R⁵ is each and independently as defined for R¹ above and p is 0, 1 or 2;

20

(C₁-C₂ alkyl)-(C₆-C₁₀ aryl); or (C₁-C₂ alkyl)heteroaryl, the heteroaryl moieties having from 5 to 10 atoms selected from any of C, S, N and O, and where the aryl or heteroaryl may optionally and independently be substituted by 1 or 2 substituents independently selected from any of hydrogen, CH₃, CONR⁵R⁴, COOR⁵, COR⁵, (CH₂)_qNR⁵R⁴,

$(CH_2)_qCH_3(CH_2)_qSOR^5R^4$, $(CH_2)_qSO_2R^5$, $(CH_2)_qSO_2NR^5$, and $(CH_2)_qOR^4$, wherein R^4 and R^5 is each and independently as defined for R^1 above and q is 0, 1 or 2;

A is



10

wherein R^6 , R^7 , R^8 , R^9 , R^{10} , R^{11} , R^{12} , R^{13} , R^{14} , R^{15} , R^{16} , R^{17} , and R^{18} is each and independently as defined for R^1 above and wherein the phenyl ring of each A substituent may be optionally and independently substituted by 1 or 2 substituents Z^1 and Z^2 which are each and independently selected from hydrogen, CH_3 , $(CH_2)_rCF_3$, halogen, $CONR^2R^3$, CO_2R^2 , COR^2 , $(CH_2)_rNR^2R^3$, $(CH_2)_rCH_3(CH_2)_rSOR^2$, $(CH_2)_rSO_2R^2$ and $(CH_2)_rSO_2NR^2R^3$ wherein R^2 and R^3 is each and independently as defined for R^1 above and wherein r is 0, 1, or 2; X is O, S or NR^{19} where R^{19} is as defined for R^1 ,

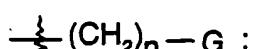
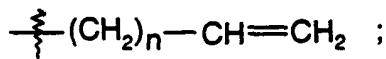
B is a substituted or unsubstituted aromatic, heteroaromatic, hydroaromatic or heterohydroaromatic moiety having from 5 to 10 atoms selected from any of C, S, N and O, optionally and independently substituted by 1 or 2 substituents independently selected from hydrogen, CH₃, (CH₂)_tCF₃, halogen, (CH₂)_tCONR⁵R⁴, (CH₂)_tNR⁵R⁴,

5 (CH₂)_tCOR⁵, (CH₂)_tCOOR⁵, OR⁵, (CH₂)_tSOR⁵, (CH₂)_tSO₂R⁵, and (CH₂)_tSO₂NR⁵R⁴, wherein R⁴ and R⁵ is each and independently as defined for R¹ above, and t is 0, 1, 2 or 3;

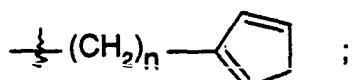
10 Within the scope of the invention are also pharmaceutically acceptable salts of the compounds of the formula (I), as well as isomers, hydrates, isoforms and prodrugs thereof.

Preferred compounds according to the invention are compounds of the formula (I) wherein

R¹ is selected from benzyl;

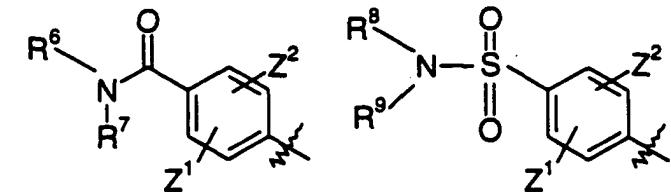


where G is a hydroaromatic or a heteroaromatic group having 5 or 6 atoms, and where the heteroatoms are selected from O, S and N; and

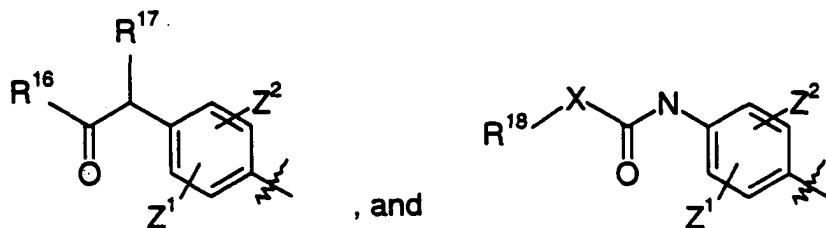


20 and wherein n = 0 or 1;

A is selected from anyone of



5



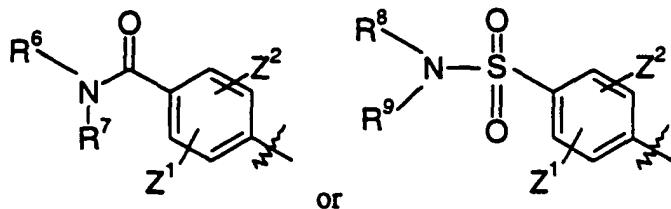
wherein R⁶, R⁷, R⁸, R⁹, R¹⁶, R¹⁷ and R¹⁸ is each and independently as defined for R¹ above; and Z¹, Z² and X is each and independently as defined above;

10 B is selected from phenyl, naphthyl, indolyl, benzofuranyl, dihydrobenzofuranyl, benzothiophenyl, pyrryl, furanyl, quinolinyl, isoquinolinyl, cyclohexyl, cyclohexenyl, cyclopentyl, cyclopentenyl, indanyl, indenyl, tetrahydronaphthyl, tetrahydroquinyl, tetrahydroisoquinolinyl, tetrahydrofuranyl, pyrrolidinyl, and indazolinyl, each optionally and independently substituted by 1 or 2 substituents independently
 15 selected from hydrogen, CH₃, CF₃, halogen, -(CH₂)_tCONR⁵R⁴, -(CH₂)_tNR⁵R⁴, -(CH₂)_tCOR⁵, -(CH₂)_tCO₂R⁵, and -OR⁵, wherein t is 0 or 1, and wherein R⁴ and R⁵ are as defined above.

Especially preferred compounds are compounds of the formula (I) wherein

5 R¹ is (C₁-C₂ alkyl)phenyl and hydrogen;

s A is



wherein R⁶, R⁷, R⁸, R⁹, is each an ethylene group; and Z¹ and Z², are as defined above;

B is phenyl or naphthalene; and

10 m and n is each 1, or m is 1 and n is 0.

The substituents A and B respectively, may optionally be substituted at any position of the ring.

15 By "halogen" we mean chloro, fluoro, bromo and iodo.

By "aryl" we mean an aromatic ring having from 6 to 10 carbon atoms, such as phenyl and naphthyl.

20 By "heteroaryl" we mean an aromatic ring in which one or more of the from 5-10 atoms in the ring are elements other than carbon, such as N, S and O.

By "hydroaromatic" we mean a partly or fully saturated aromatic ring structure having from 5-10 carbon atoms in the ring.

By "heterohydroaromatic" we mean a partly or fully saturated aromatic ring structure in which one or more of the from 5-10 atoms in the ring are elements other than carbon, such as N, S and O.

5 By "isomers" we mean compounds of the formula (I), which differ by the position of their functional group and/or orientation. By "orientation" we mean stereoisomers, diastereoisomers, regioisomers and enantiomers.

10 By "isoforms" we mean compounds of the formula (I) which differ by their crystal lattice, such as crystalline compound and amorphous compounds.

15 By "prodrug" we mean pharmacologically acceptable derivatives, e.g. esters and amides, such that the resulting biotransformation product of the derivative is the active drug. The reference by Goodman and Gilmans, The Pharmacological basis of Therapeutics, 8th ed., McGraw-Hill, Int. Ed. 1992, "Biotransformation of Drugs, p. 13-15, describing prodrugs generally, is hereby incorporated.

20 The novel compounds of the present invention are useful in therapy, especially for the treatment of various pain conditions such as chromic pain, acute pain, cancer pain, pain caused by rheumatoid arthritis, migraine, visceral pain etc. This list should however not be interpreted as exhaustive.

25 Compounds of the invention are useful as immunomodulators, especially for autoimmune diseases, such as arthritis, for skin grafts, organ transplants and similar surgical needs, for collagen diseases, various allergies, for use as anti tumour agents and anti viral agents.

30 Compounds of the invention are useful in disease states where degeneration or dysfunction of opioid receptors is present or implicated in that paradigm. This may involve the use of isotopically labelled versions of the compounds of the invention in diagnostic techniques and imaging applications such as positron emission tomography (PET).

Compounds of the invention are useful for the treatment of diarrhoea, depression, urinary incontinence, various mental illnesses, cough, lung oedema, various gastro-intestinal disorders, spinal injury and drug addiction, including the treatment of alcohol, nicotine, opioid and other drug abuse and for disorders of the sympathetic nervous system for example hypertension.

Compounds of the invention are useful as an analgesic agent for use during general anaesthesia and monitored anaesthesia care. Combinations of agents with different properties are often used to achieve a balance of effects needed to maintain the anaesthetic state (eg. Amnesia, analgesia, muscle relaxation and sedation). Included in this combination are inhaled anaesthetics, hypnotics, anxiolytics, neuromuscular blockers and opioids.

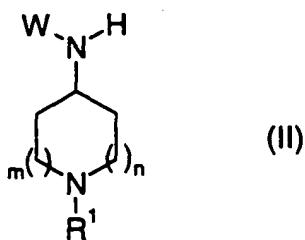
The compounds of the present invention in isotopically labelled form are useful as a diagnostic agent.

Also within the scope of the invention is the use of any of the compounds according to the formula (I) above, for the manufacture of a medicament for the treatment of any of the conditions discussed above.

A further aspect of the invention is a method for the treatment of a subject suffering from any of the conditions discussed above, whereby an effective amount of a compound according to the formula (I) above, is administered to a patient in need of such treatment.

25 Methods of preparation

Compounds of the formula (I), as described above, may be obtained by the arylation of an amine of formula (II)



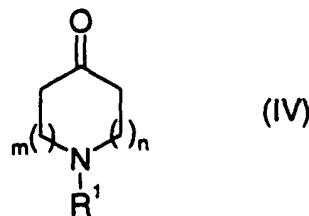
wherein R^1 , m and n are as defined in formula (I) above, and W is A or B as defined in formula (I) above, by an arylating agent of formula (III)

$W-Z$ (III)

wherein W is A or B as defined in formula (I) above, and Z is a suitable substituent, i.e. a reactive component suitable to be used in the defined process, which will be appreciated by a person skilled in the art, preferably halogen, triflate ($CF_3SO_3^-$), mesylate ($CH_3SO_3^-$), tosylate ($CH_3(C_6H_4)SO_3^-$), tributyltin, triacetoxylead, diarylbismuth, borate ($B(OH)_2$), 10 cuprate or other such group known in the art. The arylation may be catalyzed by metals, preferably Cu, Ni, Pd or suitable salts, complexes, oxides or hydroxides thereof. The 4-aminopiperidine of formula (II) above may be converted completely or partially to its corresponding anion by treatment with bases, preferably triethylamine, 4-dimethylaminopyridine, K_2CO_3 , NaOH, NaH, lithium diisopropylamide, sodium *tert*-butoxide or 15 the like, prior or during the arylation process. The reaction may be performed in the presence of complexing reagents, preferably triphenylphosphine, triphenylarsine, dibenzylideneacetone, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl, 1,1'-bis(diphenylphosphino)-ferrrocene, oxygen or other such compounds known in the art. The reaction may optionally be performed in the presence of one or more solvents such as toluene, dichloromethane, 20 tetrahydrofuran, dimethylformamide, dioxane, acetonitrile or dimethylsulfoxide, or in solvent mixtures.

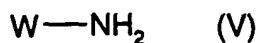
R^1 and the substituents on A and B of compound (I) as defined above, may be modified after or during the preparation of (I) from (II) and (III) by methods known in the art, for 25 example reduction, oxidation and alkylation.

The amine of formula (II) may be prepared by reductive amination of a ketone of the formula (IV)



5

wherein R¹, R², R³, m and n are as defined in formula (I) above, with a substituted arylamine (V)



10

wherein W is as defined in formula (II) above.

The reductive amination may be performed in a one or a two stage process involving a Brønstedt or a Lewis acid and a reducing agent. Suitable acids are sulphuric acid, 15 polyphosphoric acid, 4-toluenesulphonic acid, titanium *iso*-propoxide, aluminium trichloride, boron trifluoride diethyl etherate, or the like. Suitable reducing agents are hydrogen in the presence of a catalyst, preferably Pd, Pd-C, Pd(OH)₂, PtO₂, Rh-C or Raney-Nickel, sodium borohydride, sodium cyanoborohydride, lithium aluminumhydride, diborane, di-*iso*-butylaluminiumhydride, or the like. The reaction may be performed in the 20 presence of one or more solvents which may be organic or inorganic, such as toluene, dichloromethane, ethers, alcohols, acetic acid, water, or in solvent mixtures.

R¹ and the substituents on W of compound (II), as defined above, may be modified after or during preparation of (I) from (II) and (III), by methods known in the art, for example 25 reduction, oxidation and alkylation, after or during the preparation of (II) from (IV) and (V).

Compounds of formula (III), (IV) and (V) may be commercially available, prepared by literature procedures or prepared by methods known in the art.

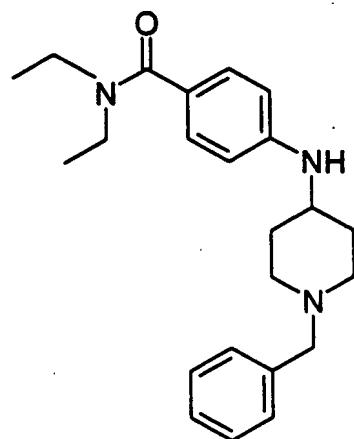
The invention will now be described in more detail by way of the following examples,
5 which should not in any way be regarded as limiting the invention.

EXAMPLES

EXAMPLE 1

10

(i) Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (compound 1)



(1)

15

Ti(O*i*-Pr)₄ (14.8 mL, 50 mmol) was added to a mixture of 4-amino-(N,N-diethyl)-benzamide (4.81 g, 25 mmol) and 1-benzyl-4-piperidone (6.95 mL, 37.5 mmol) at room temperature. The mixture was sonicated at 40 °C for 2 h, and stirred at 60 °C for 15 h. The mixture was cooled in an ice-bath and EtOH (100 mL) and NaBH₄ pellets (3.5 g, 91 mmol) were added. After stirring for 1 h at 0 °C and 20 h at room temperature, 1M NH₄OH (50 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂

(100 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the aqueous layer extracted with CH₂Cl₂ (100 mL) and the combined organic phases washed with NaHCO₃ (aq., sat., 100 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) and crystallisation (PhMe) to give the title compound **1** (7.48 g, 82%) as a beige solid.

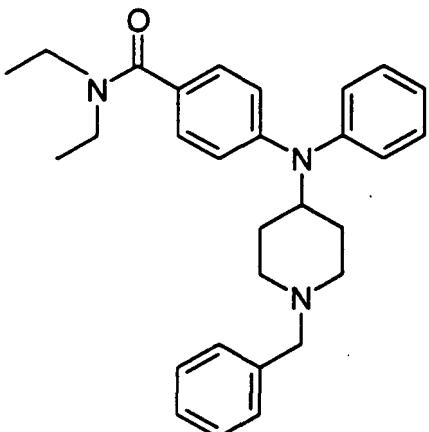
IR (KBr): 3343, 2939, 1608, 1528, 1459, 1422, 1339, 1285, 1174, 1091, 981, 827, 735 cm⁻¹.

¹H NMR (CDCl₃): 7.32 (d, 4H), 7.26 (t, 1H), 7.23 (d, 2H), 6.54 (d, 2H), 3.72 (broad s, 1H), 3.53 (s, 2H), 3.42 (broad d, 4H), 3.32 (broad s, 1H), 2.84 (d, 2H), 2.15 (t, 2H), 2.02 (d, 2H), 1.48 (q, 2H), 1.17 (t, 6H).

¹³C NMR (CDCl₃): 171.7, 148.1, 138.3, 129.1, 128.5, 128.2, 127.0, 125.3, 112.2, 63.1, 52.2, 49.8, 41.5 (broad), 32.4, 13.6 (broad).

An analytical sample was prepared by recrystallisation from PhMe.
Anal. calcd. for C₂₃H₃₁N₃O: C, 75.58; H, 8.55; N, 11.50.
Found: C, 75.58; H, 8.63; N, 11.31.

(ii) Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-anilin]-N,N-diethyl benzamide (compound 2)



(2)

A mixture of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (**compound 1**) (0.58 g, 1.59 mmol), Ph₃Bi (0.84 g, 1.90 mmol) and Cu(OAc)₂ (0.43 g, 2.38 mmol) in PhMe (25 mL) was heated at 110 °C for 15 h. Ph₃Bi (0.84 g, 1.90 mmol) and Cu(OAc)₂ (0.43 g, 2.38 mmol) was added and the mixture was stirred at reflux for 6 h. Ph₃Bi (0.84 g, 1.90 mmol) and Cu(OAc)₂ (0.43 g, 2.38 mmol) was added, the mixture stirred at reflux for 15 h, allowed to cool and quenched with 1M NH₄OH (5 mL). The mixture was stirred at room temperature for 30 min, diluted with EtOAc (25 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the deep blue aqueous layer extracted with EtOAc (25 mL) and the combined organic phases washed with H₂O (50 mL) and brine (25 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) to give the title compound **2** (0.33 g, 47%) as a colorless oil.

¹H NMR (CDCl₃): 7.53 (t, 2H), 7.29-7.18 (m, 8H), 7.01 (d, 2H), 6.58 (d, 2H), 3.85 (t, 1H), 3.49 (s, 2H), 3.42 (d, 4H), 2.95 (d, 2H), 2.11 (t, 2H), 1.92 (d, 2H), 1.51 (q, 2H), 1.17 (t, 6H).

20

¹³C NMR (CDCl₃): 171.5, 149.0, 143.6, 138.2, 129.5, 129.1, 128.7, 128.2, 128.1, 127.0, 126.7, 125.4, 116.0, 63.1, 55.5, 53.3, 40 (broad), 30.7, 13 (broad).

An analytical sample was obtained as a hydrochloride by adding a solution of the free base in ether/EtOH to ice cold diluted etheral HCl.

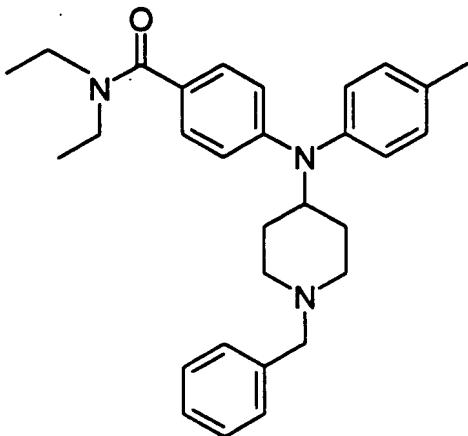
IR (KBr): 3423, 2975, 2934, 2529, 1606, 1458, 1285, 1094, 750, 705 cm⁻¹.

Anal. calcd. for C₂₉H₃₅N₃O*HCl*H₂O: C, 70.21; H, 7.72; N, 8.47

Found: C, 70.02; H, 7.61; N, 8.35

EXAMPLE 2

Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-4-methyl-anilino]-N,N-diethyl benzamide (compound 3)



(3)

A mixture of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (**compound 1**) (0.37 g, 1.00 mmol), tri-4-tolylbismuth (1.59 g, 3.30 mmol) and Cu(OAc)₂ (0.54g, 3.00 mmol) in PhMe (20 mL) was heated at reflux for 16 h. The mixture was allowed to cool and quenched with H₂O (2 mL). The mixture was stirred for 1 h, diluted with EtOAc (25 mL) and filtered through a pad of Celite[®]. The layers in the filtrate were separated, the aqueous layer extracted with EtOAc (25 mL) and the combined organic phases washed with H₂O (50 mL) and brine (25 mL) and dried over MgSO₄. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, CH₂Cl₂ to 8% MeOH/CH₂Cl₂) to give the title compound **3** (0.09 g, 20%) as a colorless oil.

10

¹H NMR (CDCl₃): 7.30-7.16 (m, 9H), 6.94 (d, 2H), 6.52 (d, 2H), 3.83 (t, 1H), 3.48 (s, 2H), 3.42 (d, 4H), 2.93 (d, 2H), 2.36 (s, 3H), 2.07 (t, 2H), 1.90 (d, 2H), 1.50 (q, 2H), 1.16 (t, 6H).

15

¹³C NMR (CDCl₃): 171.6, 149.7, 140.4, 138.2, 136.1, 130.2, 130.2, 129.0, 128.1, 128.0, 126.9, 125.3, 113.8, 63.0, 55.4, 53.3, 41 (broad), 30.5, 21.0, 13 (broad).

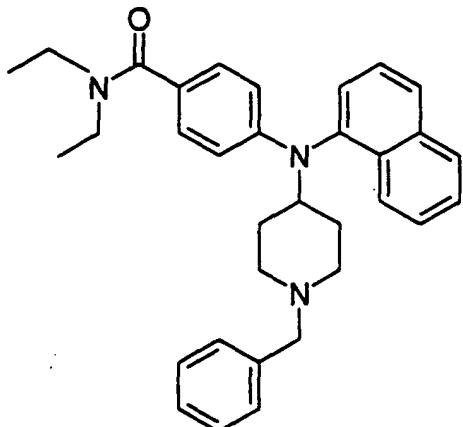
An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

20

IR (KBr): 2936, 2528, 1605, 1510, 1457, 1428, 1284, 1094, 952, 742, 701 cm⁻¹.

Anal. calcd. for C₃₀H₃₇N₃O*HCl*0.5 H₂O: C, 71.91; H, 7.84; N, 8.39

Found: C, 71.75; H, 7.83; N, 8.32

EXAMPLE 3**Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-1-naphthylaminol-N,N-diethyl benzamide (compound 4)**

(4)

5

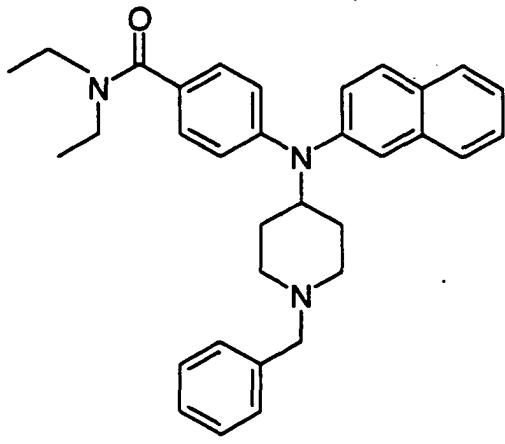
A mixture of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (**compound 1**) (0.37 g, 1.00 mmol), tri-1-naphthylbismuth (0.53 g, 1.20 mmol) and Cu(OAc)₂ (0.27 g, 1.50 mmol) in PhMe (20 mL) was heated at reflux for 17 h. Tri-1-naphthylbismuth (0.53 g, 1.20 mmol) and Cu(OAc)₂ (0.27 g, 1.50 mmol) was added at room temperature. The mixture was stirred at reflux for 22 h, allowed to cool and quenched with 1M NH₄OH (5 mL). The mixture was stirred for 30 min, diluted with EtOAc (25 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the deep blue aqueous layer extracted with EtOAc (25 mL) and the combined organic phases washed with H₂O (50 mL) and brine (25 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) to give the title compound **4** (0.41 g, 83%) as a brownish solid.

IR (KBr): 2939, 2796, 1619, 1511, 1456, 1420, 1346, 1282, 1180, 1099, 783 cm⁻¹.

¹H NMR (CDCl_3): 7.87 (t, 2H), 7.79 (d, 1H), 7.51 (t, 1H), 7.46 (t, 1H), 7.37 (t, 1H), 7.31 (d, 1H), 7.28-7.19 (m, 5H), 7.17 (d, 2H), 6.41 (d, 2H), 4.08 (t, 1H), 3.46 (s, 2H), 3.40 (d, 4H), 2.89 (d, 2H), 2.11 (t, 2H), 2.08 (broad s, 2H), 1.50 (broad s, 2H), 1.14 (t, 6H).

⁵ ^{13}C NMR (CDCl_3): 171.7, 149.8, 138.9, 138.1, 135.0, 133.4, 129.1, 129.0, 128.3, 128.2, 128.1, 128.0, 127.0, 126.4, 126.2, 126.1, 124.6, 124.5, 112.2, 63.0, 56.5, 53.3, 40 (broad), 30.4, 13.5 (broad).

An analytical sample was obtained by recrystallization from EtOH
10 Anal. calcd. for $C_{33}H_{37}N_3O$: C, 80.61; H, 7.59; N, 8.55
Found: C, 80.48; H, 7.41; N, 8.52

EXAMPLE 4**Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-2-naphthylamino]-N,N-diethyl benzamide (compound 5)**

(5)

5

A mixture of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (**compound 1**) (0.67 g, 1.83 mmol), tri-2-naphthylbismuth (0.97 g, 2.20 mmol) and Cu(OAc)₂ (0.50 g, 2.75 mmol) in PhMe (35 mL) was heated at reflux for 15 h. Tri-2-naphthylbismuth (0.97 g, 2.20 mmol) and Cu(OAc)₂ (0.50 g, 2.75 mmol) was added. and the mixture was stirred at reflux for 22 h. Tri-2-naphthylbismuth (0.97 g, 2.20 mmol) and Cu(OAc)₂ (0.50 g, 2.75 mmol) was added. After reflux for 70 h the mixture was allowed to cool and quenched with 1M NH₄OH (10 mL). The mixture was stirred for 30 min, diluted with EtOAc (35 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the deep blue aqueous layer extracted with EtOAc (35 mL) and the combined organic phases washed with H₂O (75 mL) and brine (35 mL) and dried over MgSO₄. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) to give the title compound **5** (0.63 g, 70%) as a brown oil that solidified on standing.

20 IR (KBr): 2935, 2807, 1614, 1510, 1424, 1284 cm⁻¹.

¹H NMR (CDCl₃): 7.83-7.78 (m, 2H), 7.74 (d, 1H), 7.48-7.42 (m, 3H), 7.27-7.21 (m, 7H), 7.10 (dd, 1H), 6.66 (d, 2H), 3.94 (t, 1H), 3.48 (s, 2H), 3.43 (broad s, 4H), 2.94 (d, 2H), 2.14 (t, 2H), 1.99 (s, 2H), 1.57 (m, 2H), 1.17 (t, 6H).

5 ¹³C NMR (CDCl₃): 171.4, 148.8, 141.3, 138.1, 134.2, 131.2, 129.2, 129.0, 128.1, 128.0, 127.5, 127.4, 127.2, 126.9, 126.2, 125.4, 125.3, 116.8, 63.0, 55.8, 53.3, 41 (broad), 30.7, 13.6 (broad).

An analytical sample was obtained from MeOH.

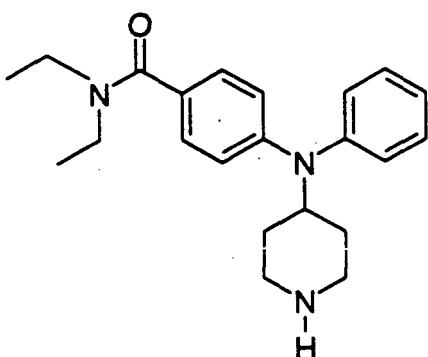
10

Anal. calcd. for C₃₃H₃₇N₃O: C, 80.61; H, 7.59; N, 8.55

Found: C, 80.35; H, 7.59; N, 8.46

EXAMPLE 5

15 Preparation of N,N-diethyl-4-(N-piperidin-4-yl-anilino) benzamide (compound 6)



(6)

20 A solution of 4-[N-(1-benzyl-piperidin-4-yl)-anilino]-N,N-diethyl benzamide (compound 2) (1.21 g, 2.74 mmol) in MeOH (25 mL) was hydrogenated at 60 psi for 4 d in the presence of a catalytic amount of Pd(OH)₂ on carbon. The mixture was filtered through a pad of

Celite, concentrated and the residue purified by chromatography (gradient, CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to CH₂Cl₂/ MeOH/ NH₄OH (aq., conc.) (80:18:2) to give the title compound **6** (0.62 g, 64%) as a colorless oil.

⁵ ¹H NMR (CDCl₃): 7.37 (t, 2H), 7.25-7.22 (m, 3H), 7.01 (d, 2H), 6.61 (d, 2H), 3.98 (t, 1H), 3.42 (broad d, 4H), 3.17 (d, 2H), 2.78 (t, 2H), 2.00 (d, 2H), 1.71 (broad s, 1H), 1.41 (q, 2H), 1.18 (t, 6H).

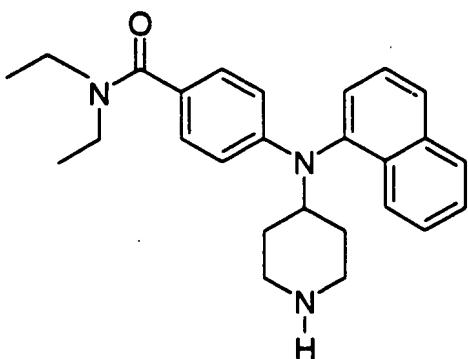
¹⁰ ¹³C NMR (CDCl₃): 171.4, 148.2, 143.1, 129.8, 128.0, 127.9, 127.7, 125.7, 116.9, 53.5, 44.4, 41 (broad), 28.7, 13.5 (broad).

An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

15 IR (KBr): 3426, 3359, 2936, 2722, 1603, 1473, 1281, 1091, 708, 503 cm⁻¹.
 Anal. calcd. for C₂₂H₂₉N₃O*HCl*H₂O: C, 65.09; H, 7.95; N, 10.35
 Found: C, 65.37; H, 7.94; N, 10.38

EXAMPLE 6

**20 Preparation of N,N-diethyl-4-([N-piperidin-4-yl]-1-naphthylamino benzamide
(compound 7)**



(7)

A solution of 4-[N-(1-benzyl-piperidin-4-yl)-1-naphthylamino]-N,N-diethyl benzamide (**compound 4**) (0.22 g, 0.45 mmol) in EtOH (20 mL) was hydrogenated at 60 psi for 64 h in the presence of a catalytic amount of Pd(OH)₂ on carbon. The mixture was filtered through a pad of Celite[®], concentrated and the residue purified by chromatography (gradient, CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to CH₂Cl₂/MeOH/ NH₄OH (aq., conc.) (80:18:2)) to give the title **compound 7** (0.12 g, 67%) as a colorless oil that solidified on standing.

IR (KBr): 2942, 1609, 1512, 1448, 1280 cm⁻¹.

10

¹H NMR (CDCl₃): 7.86 (t, 2H), 7.80 (d, 1H), 7.50 (t, 1H), 7.45 (t, 1H), 7.36 (t, 1H), 7.30 (d, 1H), 7.18 (d, 2H), 6.42 (d, 2H), 4.20 (t, 1H), 3.40 (broad d, 4H), 3.06 (d, 2H), 2.79-2.63 (m, 3H), 2.03 (broad s, 2H), 1.39 (broad s, 2H), 1.14 (t, 6H).

15

¹³C NMR (CDCl₃): 171.6, 149.4, 138.7, 134.8, 133.3, 128.7, 128.2, 128.1, 127.9, 126.3, 126.1, 126.0, 124.4, 124.2, 112.1, 56.1, 46.0, 41 (broad), 31.3, 13.4 (broad).

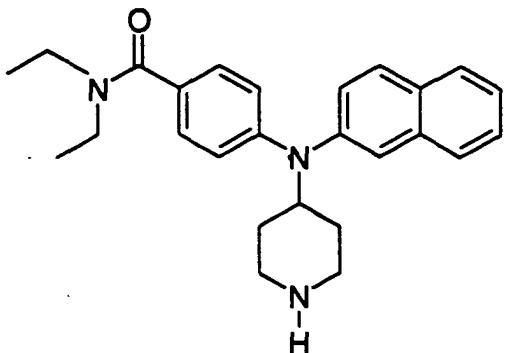
Anal. calcd. for C₂₆H₃₁N₃O*1.25 H₂O: C, 73.64; H, 7.96; N, 9.91

Found: C, 73.77; H, 7.54; N, 9.96

20

EXAMPLE 7**Preparation of N,N-diethyl-4-([N-piperidin-4-yl]-2-naphthylamino benzamide
(compound 8)**

5



(8)

(1-chloroethyl) chloroformate (58 μ L, 0.53 mmol) was added to a solution of 4-[N-(1-benzyl-piperidin-4-yl)-2-naphthylamino]-N,N-diethyl benzamide (**compound 5**) (105 mg, 0.21 mmol) in dichloroethane (2.5 mL) at room temperature. The mixture was heated at reflux for 17h and was then allowed to cool to room temperature and concentrated. Methanol (2.5 mL) was added and the mixture was heated at reflux for 2.5 h, allowed to cool and concentrated. The residue was partitioned between CH_2Cl_2 (10 mL) and 1M NH_4OH (10 mL). The layers were separated and the organic phase was washed with H_2O (10 mL) and brine (10 mL) and dried over K_2CO_3 . The mixture was filtered, concentrated and the residue purified by chromatography (gradient, CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{NH}_4\text{OH}$ (aq., conc.) (85:13.5:1.5) and HPLC (LiChroPrep RP-18, elution with increasing amounts of 0.1% TFA/MeCN in 0.1% TFA/ H_2O) to give the title compound **8** (30 mg) as the trifluoroacetate.

10

IR (neat): 3420, 1680, 1600 cm^{-1} .

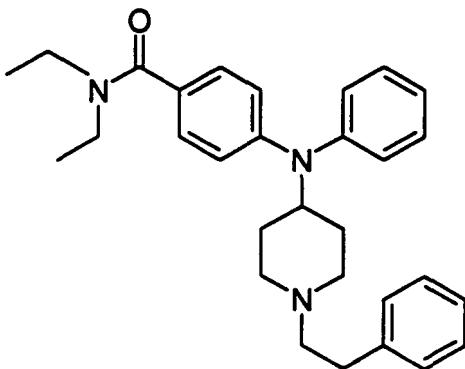
15

¹H NMR (CDCl₃, TFA salt) δ: 1.10 (6H, m), 1.78 (2H, m), 2.10 (2H, m, CH(CH)CH(N)CH(CH)), 3.00 (2H, m, CH(CH)NHCH(CH)), 3.35 (6H, m), 4.15 (1H, m.), 6.65 (2H, m), 7.00 (1H, m), 7.20 (2H, m), 7.40 (3H, m), 7.75 (3H, m, Ar).

5

EXAMPLE 8

Preparation of 4-[N-(1-[2-phenylethyl]-piperidin-4-yl)-anilino]-N,N-diethyl-benzamide (compound 9)



(9)

10

(2-bromoethyl)benzene (0.18 mL, 1.30 mmol) was added with stirring to an ice-cold solution of N, N-diethyl-4-(N-piperidin-4-yl-anilino) benzamide (**compound 6**) (0.21g, 0.59 mmol), Et₃N (0.10 mL, 0.75 mmol) and a catalytic amount of 4-dimethylaminopyridine in CH₂Cl₂ (5 mL). The stirred mixture was allowed to attain room temperature over 5 h, heated at reflux for 16 h, allowed to cool to room temperature, diluted with CH₂Cl₂ (10 mL) and washed with H₂O (15 mL), brine (15 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to PhMe / Me₂CO (1:2)) to give the title **compound 9** (0.11 g, 40%) as a colorless oil which solidified on standing.

IR (KBr): 2928, 1612, 1504, 1437, 1280, 1980, 754 cm⁻¹.

¹H NMR (CDCl₃): 7.36 (t, 2H), 7.30-7.15 (m, 8H), 7.01 (d, 2H), 6.61 (d, 2H), 3.88 (t, 1H),

3.42 (broad d, 4H), 3.08 (d, 2H), 2.76 (m, 2H), 2.57 (m, 2H), 2.17 (t, 2H), 1.97 (d, 2H),

5 1.55 (q, 2H), 1.18 (t, 6H).

¹³C NMR (CDCl₂): 171.5, 149.0, 143.5, 140.3, 129.6, 128.6, 128.6, 128.4, 128.1, 126.8,

126.1, 125.4, 116.1, 60.6, 55.5, 53.5, 41 (broad), 33.9, 30.7, 13.6 (broad).

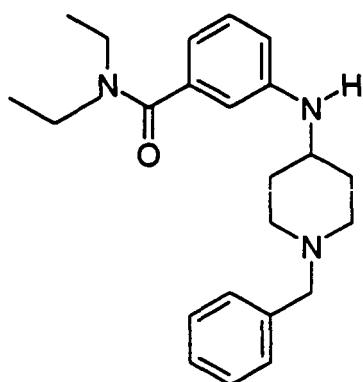
10 Anal. calcd. for C₃₀H₃₇N₃O*0.2 C₃H₆O: C, 78.66; H, 8.24; N, 8.99.

Found: C, 78.55; H, 7.75; N, 8.91.

EXAMPLE 9

15

(i) Preparation of 3-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide
(compound 10)



(10)

20

Ti(O*i*-Pr)₄ (0.70 mL, 2.37 mmol) was added to a mixture of 3-amino-(*N,N*-diethyl) benzamide (150 mg, 0.78 mmol) and 1-benzyl-4-piperidone (0.18 mL, 0.97 mmol) at room temperature. The mixture was sonicated at 40 °C for 6 h, and stirred at room temperature for 15 h. The mixture was cooled in an ice-bath and EtOH (5 mL) and NaBH₄ (75 mg, 1.98 mmol) were added. After stirring for 1 h at 0 °C and 2 d at room temperature, 2M NH₄OH (5 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂ (10 mL) and filtered through Celite®. The layers in the filtrate were separated, the aqueous layer extracted with CH₂Cl₂ (3x10 mL). The combined organic phases were washed with 10% HCl (2x15 mL). The pH in the combined aqueous extracts was adjusted to 10 with 2N NaOH, and extracted with CH₂Cl₂ (3x10 mL). The combined organic extracts were dried over Na₂SO₄, filtered, concentrated and the residue purified by chromatography (9:1:0.1 EtOAc:heptane: Et₃N) to give the title compound **10** (173 mg, 61 %) as a pale yellow thick oil.

15 IR (neat): 3333, 1612 cm⁻¹.

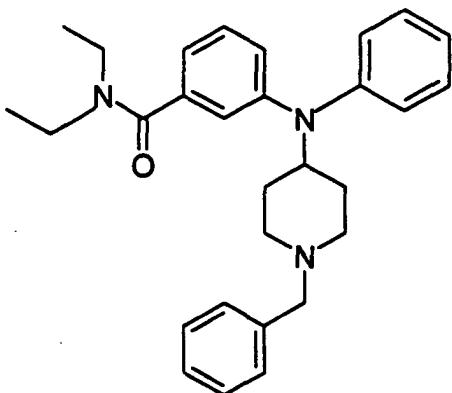
¹H NMR (CDCl₃): 7.40-7.10 (7H, m), 6.55 (2H, m), 3.50 (4H, m), 3.22 (4H, m), 2.80 (2H, m), 2.12 (2H, m), 2.00 (2H, m), 1.43 (2H, m), 1.20 (3H, m), 1.05 (3H, m).

20 ¹³C NMR (CDCl₃): 171.6, 147.1, 138.3, 138.2, 129.1, 129.0, 128.1, 126.9, 114.6, 113.8, 110.6, 63.0, 52.2, 49.8, 43.1, 38.9, 32.4, 14.1, 12.8.

Anal. calcd. for C₂₃H₃₁N₃O*HCl*2.1 H₂O: C, 63.07; H, 8.28; N, 9.59.

Found: C, 63.19; H, 7.94; N, 9.25.

**(ii) Preparation of 3-[N-(1-benzyl-piperidin-4-yl)-anilino]-N,N-diethyl benzamide
(compound 11)**



5 (11)

A mixture of 3-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzamide (compound 10) (360 mg, 0.98 mmol), Ph₃Bi (1.10 g, 2.50 mmol), and Cu(OAc)₂ (0.45 g, 2.48 mmol) in toluene (10 mL) was heated at 110 °C for 12 h. Another portion of Cu(OAc)₂ (0.45 g) was added and the mixture was heated at 110 °C for an additional 12 h and allowed to cool to room temperature. Water (10 mL) was added and the mixture filtered through Celite®. The layers in the filtrate were separated, and the organic phase was washed with water, brine, dried over Na₂SO₄ and concentrated. Chromatography of the residue (9:1 EtOAc/heptane) gave the title compound 11 (255 mg, 59%) as a pale yellow thick oil.

15

IR (neat): 3056, 3010, 2937, 2810, 1629 cm⁻¹.

¹H NMR (CDCl₃): 7.40-6.60 (14H, m), 3.80 (1H, m), 3.43 (4H, bs), 3.20 (2H, bs), 2.90 (2H, m), 2.05 (2H, m), 1.90 (2H, m), 1.48 (2H, m), 1.20 (3H, bs), 1.00 (3H, bs).

20

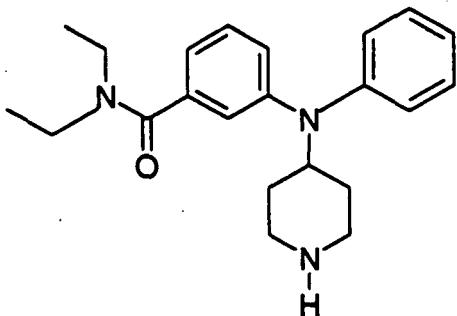
¹³C NMR (CDCl₃): 171.3, 147.3, 144.4, 138.1, 129.3, 129.0, 128.0, 126.9, 126.4, 123.9, 119.7, 117.5, 116.5, 62.9, 55.3, 53.2, 43.0, 39.0, 30.6, 14.0, 12.7.

Anal. calcd. for C₂₉H₃₅N₃O 1.25HCl*0.5 H₂O: C, 70.15; H, 7.41; N, 8.47; Cl, 8.94.
 Found: C, 69.69; H, 7.34; N, 8.25; Cl, 8.96.

5

EXAMPLE 10

Preparation of N,N-diethyl-3-(N-piperidin-4-yl-anilino) benzamide (compound 12)



(12)

10

A solution of 3-[N-(1-benzyl-piperidin-4-yl)-anilino]-N,N-diethyl benzamide (**compound 11**) (102 mg, 0.23 mmol) in EtOH (15 mL) was hydrogenated at 40 psi in the presence of a catalytic amount of Pd(OH)₂ on carbon for 2 h. The mixture was filtered through Celite[®] and concentrated. The residue was purified by chromatography (9:1:0.5 EtOAc/ heptane/Et₃N) to yield the title **compound 12** (50 mg, 81 %) as a pale yellow viscous oil.

IR: (HCl-salt, neat) 3421, 1597, 1494 cm⁻¹.

¹H NMR (CDCl₃): 7.80-6.50 (9H, m), 4.00 (1H, m), 3.21 (2H, bs), 3.30 (2H, m), 3.20 (2H, bs), 2.90 (2H, m), 2.05 (2H, m), 1.70 (2H, m), 1.17 (3H, bs), 1.00 (3H, bs).

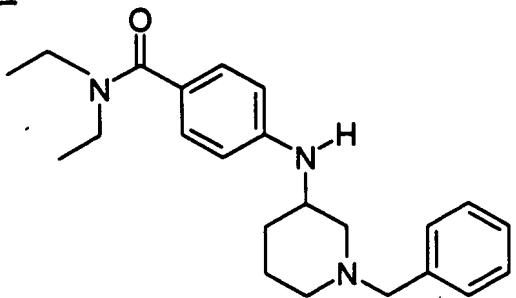
¹³C NMR (CDCl₃): 171.0, 143.7, 140.7, 138.0, 129.5, 129.2, 128.2, 124.5, 119.7, 117.9, 116.5, 53.2, 43.1, 41.1, 39.0, 28.7, 14.0, 8.9.

An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

5 Anal. calcd. for $C_{28}H_{33}N_3O^*HCl \cdot 1.3 H_2O$: C, 62.30; H, 8.08; N, 9.91.
 Found: C, 62.40; H, 7.67; N, 9.80.

EXAMPLE 11

10 (i) Preparation of 4-[N-(1-benzyl-piperidin-3-yl)-amino]-N,N-diethyl benzamide
(Compound 13)



(13)

Ti(O*i*-Pr)₄ (2.2 mL, 7.45 mmol) was added to a mixture of 4-amino-(N,N-diethyl) 15 benzamide (0.36 g, 1.87 mmol) and 1-benzyl-3-piperidone (0.70 g, 3.69 mmol) at room temperature. The mixture was sonicated at 40 °C for 1 h, and stirred at room temperature for 15 h. The mixture was cooled in an ice-bath and EtOH (15 mL) and NaBH₄ (0.21 g, 5.55 mmol) were added. After stirring for 16 h at room temperature, 2M NH₄OH (15 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂ 20 (10 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the aqueous layer extracted with CH₂Cl₂ (3x15 mL). The combined organic phases were washed with 10% HCl (2x20 mL). The pH in the combined aqueous extracts was adjusted to 10 with 2N NaOH, and extracted with CH₂Cl₂ (3x10 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated. The residue was purified by

chromatography (9:1:0.1 EtOAc/heptane/ Et₃N) to give the title compound 13 (0.32 g, 47%) as a pale yellow thick oil.

IR (neat): 3320, 1736, 1608 cm⁻¹.

5

¹H NMR (CDCl₃): 7.20 (7H, m), 6.50 (2H, m), 4.22 (1H, bs), 3.60-3.30 (7H, m), 2.60 (1H, m), 2.35 (3H, m), 1.65 (2H, m), 1.50 (2H, m), 1.10 (6H, m).

¹³C NMR (CDCl₃): 171.7, 147.9, 138.1, 128.7, 128.4, 128.1, 126.9, 124.9, 112.1, 63.0,

10 58.6, 53.5, 48.3, 41.4, 28.6, 22.3, 13.4.

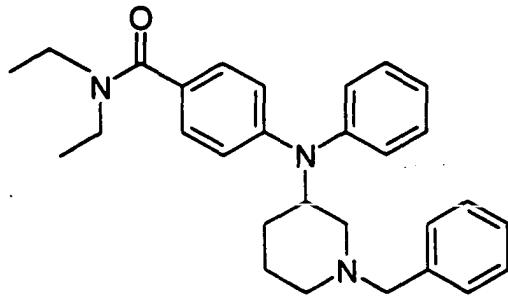
Anal. calcd. for C₂₃H₃₁N₃O*HCl*2.1 H₂O: C, 62.81; H, 8.30; N, 9.55.

Found:

C, 62.75; H, 7.94; N, 9.63.

15

(ii) Preparation of 4-[N-(1-benzyl-piperidin-3-yl)-anilino]-N,N-diethyl benzamide (compound 14).



(14)

20

A mixture of 4-[N-(1-benzyl-piperidin-3-yl)-amino]-N,N-diethyl benzamide (**compound 13**) (0.29 mg, 0.79 mmol), Ph₃Bi (0.87 g, 1.98 mmol), and Cu(OAc)₂ (0.36 g, 1.98 mmol) in toluene (5 mL) was heated at 110 °C for 12h and allowed to cool to room temperature. Water (5 mL) was added and the mixture filtered through Celite®. The layers in the filtrate were separated and the organic phase washed with water, brine, dried over Na₂SO₄ and concentrated. Chromatography of the residue (9:1 EtOAc/heptane) gave the title compound **14** (0.24 g, 67%) as a pale yellow viscous oil.

IR (neat): 3056, 3012, 2938, 2800, 1613, 1492 cm⁻¹.

10

¹H NMR (CDCl₃): 7.40-7.10 (10H, m), 6.95 (2H, d), 6.55 (2H, d), 4.05 (1H, m), 3.45 (2H, s), 3.38 (4H, bs), 3.19 (1H), 2.75 (1H, m), 1.90 (1H, m), 1.80-1.60 (4H, m), 1.12 (6H, m).

15

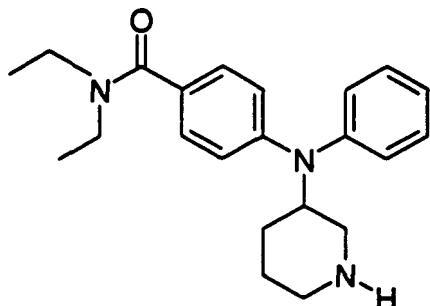
An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

¹³C NMR (CDCl₃): 171.4, 148.8, 143.4, 138.2, 129.3, 128.7, 128.6, 128.4, 128.0, 127.8, 126.8, 125.3, 123.9, 115.7, 62.9, 57.2, 54.4, 53.2, 42(b), 29.8, 25.9, 13.4(b).

20

Anal. calcd. for C₂₉H₃₅N₃O*1.25HCl*0.5 H₂O: C, 70.15; H, 7.41; N, 8.47; Cl, 8.94.

Found: C, 70.30; H, 7.30; N, 8.43; Cl, 8.34.

EXAMPLE 12**Preparation of N,N-Diethyl-4-(N-piperidin-3-yl-anilino) benzamide (Compound 15)**

5

A solution of 4-[N-(1-benzyl-piperidin-3-yl)-anilino]-N,N-diethyl benzamide (**compound 14**) (0.28 g, 0.63 mmol) in EtOH (10 mL) was hydrogenated at 30 psi in the presence of a catalytic amount of $\text{Pd}(\text{OH})_2$ on carbon for 6 h. The mixture was filtered through Celite[®], concentrated and the residue purified by chromatography (gradient, 9:1:0 to 9:1:0.5 EtOAc/heptane/Et₃N) to yield the title compound **15** (80 mg, 36%) as a pale yellow viscous oil.

IR:(neat) 3300-3500, 1609, 1464 cm^{-1} .

15

¹H NMR (CDCl_3): 7.35 (2H, m), 7.18 (3H, m), 6.95 (2H, m), 6.56 (2H, m), 4.30 (1H, bs), 4.0 (1H, m), 3.38 (4H, bs), 2.95 (1H, m), 2.35 (2H, m), 1.95 (1H, m), 1.70 (2H, m), 1.15 (2H, m), 1.10 (6H, m).

20

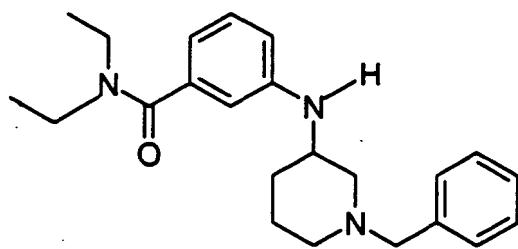
¹³C NMR (CDCl_3): 171.3, 148.5, 142.8, 129.3, 128.5, 127.7, 126.4, 125.5, 115.4, 54.1, 49.3, 45.4, 44-38(b), 29.9, 25.6, 13.3(b).

Anal. calcd. for $\text{C}_{22}\text{H}_{29}\text{N}_3\text{O}^*\text{HCl}^*\text{0.4 H}_2\text{O}$: C, 66.87; H, 7.86; N, 10.63.

Found: C, 66.85; H, 7.68; N, 10.44.

EXAMPLE 13**(i) Preparation of 3-[N-(1-benzyl-piperidin-3-yl)-amino]-N,N-diethyl benzamide**

5 **(compound 16)**



(16)

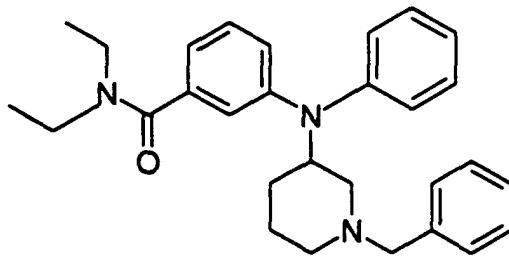
Ti(O*i*-Pr)₄ (6.2 mL, 21.0 mmol) was added to a mixture of 3-amino-(N,N-diethyl) benzamide (1.0 g, 5.2 mmol) and 1-benzyl-3-piperidone (2.0 g, 10.6 mmol) at room temperature. The mixture was sonicated at 40 °C for 2 h, and stirred at room temperature for 16 h. The mixture was cooled in an ice-bath and EtOH (30 mL) and NaBH₄ (0.60 g, 15.9 mmol) were added. After stirring for 16 h at room temperature, 2M NH₄OH (25 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂ (25 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated and the aqueous layer extracted with CH₂Cl₂ (3x25 mL). The combined organic phases were washed with 10% HCl (2x25 mL). The pH in the combined aqueous extracts was adjusted to 10 with 2N NaOH, and extracted with CH₂Cl₂ (3x25 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated. The residue was purified by chromatography (9:1:0.1 EtOAc/heptane/ Et₃N) to give the title compound 16 (1.10 g, 20 58%) as a pale yellow viscous oil.

IR (neat): 3327, 1606 1440 cm⁻¹.

¹H NMR (CDCl₃): 7.40-7.00 (7H, m), 6.60-6.40 (2H, m), 4.20 (1H, bs), 3.50 (4H, m), 3.20 (2H, bs), 2.60 (1H, m), 2.40-2.20 (3H, m), 1.70 (2H, m), 1.50 (2H, m), 1.20 (3H, bs), 1.00 (3H, bs).

5 ¹³C NMR (CDCl₃): 171.4, 146.9, 138.1, 137.9, 128.9, 128.6, 127.9, 126.7, 114.1, 113.5, 110.4, 62.8, 58.5, 53.3, 48.3, 42.9, 38.7, 28.6, 22.2, 13.9, 12.6.

10 **(ii) Preparation of 3-[N-(1-benzyl-piperidin-3-yl)-anilino]-N,N-diethyl benzamide
(compound 17)**



(17)

A mixture of 3-[N-(1-Benzyl-piperidin-3-yl)-amino]-N,N-diethyl benzamide (compound 16) (0.25 g, 0.68 mmol), Ph₃Bi (0.75 g, 1.70 mmol), and Cu(OAc)₂ (0.31 mg, 1.70 mmol) in toluene (5 mL) was heated at 110 °C for 14h and allowed to cool to room temperature. Water (5 mL) was added and the mixture was filtered through Celite®. The layers in the filtrate were separated and the organic phase was washed with water and brine, dried over Na₂SO₄ and concentrated. Chromatography of the residue (9:1 EtOAc/heptane) gave the title compound 17 (0.16 g, 52%) as a pale yellow viscous oil.

20

IR (neat): 3010, 2930, 1630, 1610 cm⁻¹.

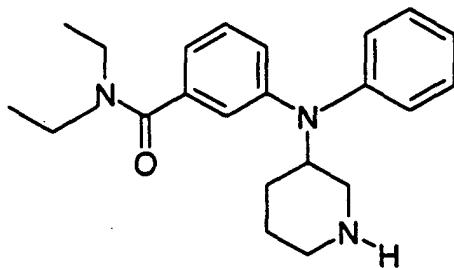
¹H NMR (CDCl₃): 7.40-6.60 (14H, m), 4.05 (1H, m), 3.45 (4H, bs), 3.18 (2H, m), 2.75 (1H, m), 1.90 (1H, m), 1.80-1.60 (4H, m), 1.18 (3H, bs), 1.00 (3H, bs).

¹³C NMR (CDCl₃): 171.3, 147.1, 144.5, 138.3, 138.1, 129.3, 129.1, 128.8, 128.1, 126.9, 125.9, 123.7, 119.8, 117.8, 116.9, 63.0, 57.5, 54.4, 53.2, 43.1, 39.0, 29.9, 25.0, 14.1, 12.8.

5 Anal. calcd. for C₂₉H₃₅N₃O*1.4HCl*0.5 H₂O: C, 69.38; H, 7.46; N, 8.37; Cl, 9.91.
Found: C, 69.11; H, 7.14; N, 8.08; Cl, 10.12.

EXAMPLE 14

Preparation of N,N-Diethyl-3-(N-piperidin-3-yl-anilino) benzamide (compound 18)



(18)

A solution of 3-[N-(1-benzyl-piperidin-3-yl)-anilino]-N,N-diethyl benzamide (**compound 17**) (50 mg, 0.11 mmol) in EtOH (5 mL) was hydrogenated at 30 psi in the presence of a catalytic amount of Pd(OH)₂ on carbon for 6 h. The mixture was filtered through Celite[®], concentrated and the residue purified by chromatography (gradient, 9:1:0 to 9:1:0.5 EtOAc/heptane/Et₃N) to yield the title compound **18** (15 mg, 36%) as a pale yellow viscous oil.

¹H NMR (CDCl_3): 7.40-6.60 (9H, m), 4.40 (1H, m), 3.60 (2H, m), 3.40 (2H, bs), 3.15 (3H, m), 2.50 (2H, m), 1.80 (2H), 1.20 (2H, m), 1.18 (3H, bs), 0.95 (3H, bs).

¹³C NMR (CDCl₃): 171.2, 146.9, 144.0, 138.0, 129.4, 126.4, 124.3, 119.7, 117.8, 116.5, 54.1, 45.1, 43.1, 39.0, 30.0, 14.0, 12.7.

An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

s IR: (neat) 3412, 1598, 1493 cm⁻¹.

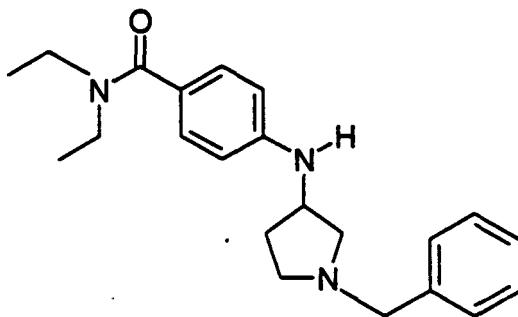
Anal. calcd. for C₂₂H₂₉N₃O*HCl*H₂O: C, 65.09; H, 7.95; N, 10.35.

Found: C, 65.03; H, 7.80; N, 10.02.

EXAMPLE 15

10

(i) Preparation of 4-[N-(1-benzyl-pyrrolidin-3-yl)-amino]-N,N-diethyl benzamide (compound 19)



(19)

Ti(O*i*-Pr)₄ (3.1 ml, 10.4 mmol) was added to a mixture of 4-amino-(N,N-diethyl)
15 benzamide (0.50 g, 2.51 mmol) and 1-benzyl-3-pyrrolidone (0.85 mL, 5.30 mmol) at room temperature. The mixture was sonicated at 40 °C for 3 h, and stirred at room temperature for 16 h. The mixture was cooled in an ice-bath and EtOH (30 mL) and NaBH₄ (0.30 g, 8.00 mmol) were added. After stirring for 16 h at room temperature, 2M NH₄OH (20 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂ (20 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the aqueous layer extracted with CH₂Cl₂ (3x20 mL). The combined organic phases were washed with 10% HCl (2x20 mL). The pH in the combined aqueous extracts was adjusted to 10 with 2N NaOH, and extracted with CH₂Cl₂ (3x20 mL). The combined organic extracts were dried over Na₂SO₄, filtered, concentrated and the residue purified by

chromatography (gradient, 9:1:0.5 to 9:0:1 EtOAc/ heptane/Et₃N) to give the title compound **19** (0.40 g, 44%) as a pale yellow viscous oil.

IR (neat): 3322, 1609, 1527, 1455 cm⁻¹.

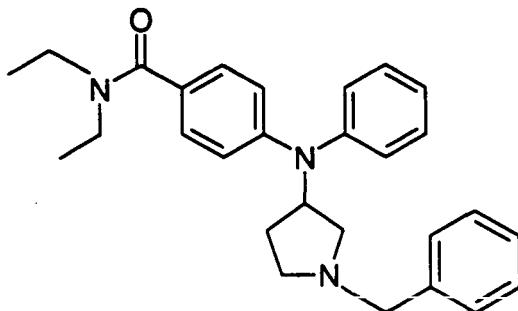
5

¹H NMR (CDCl₃): 7.40-7.10 (7H, m), 6.60-6.40 (2H, m), 4.20 (1H, m), 3.95 (1H, m), 3.55 (2H, s), 3.35 (4H, bs), 2.70 (2H, m), 2.50-2.35 (2H, m), 2.25 (1H, m), 1.60 (1H, m), 1.15 (6H, bt).

10 ¹³C NMR (CDCl₃): 171.4, 148.2, 138.4, 128.5, 128.2, 128.1, 126.7, 126.1, 113.8, 112.1, 60.5, 59.9, 52.5, 51.9, 41 (b), 32.2, 13.3.

(ii) Preparation of 4-[N-(1-benzyl-pyrrolidin-3-yl)-anilino]-N,N-diethyl benzamide

15 (**compound 20**)



(20)

A mixture of 4-[N-(1-benzyl-pyrrolidin-3-yl)-amino]-N,N-diethyl benzamide (**compound 19**) (0.40 g, 1.14 mmol), Ph₃Bi (1.25 g, 2.84 mmol), and Cu(OAc)₂ (0.52 g, 2.86 mmol) in toluene (10 mL) was heated at 110 °C for 16h and allowed to cool to room temperature. Water (5 mL) was added and the mixture was filtered through Celite[®]. The filtrate was washed with water, brine, dried over Na₂SO₄ and concentrated. Chromatography of the residue (95:5 EtOAc/MeOH) gave the title compound **20** (0.19 g, 40%) as a pale yellow viscous oil.

¹H NMR (CDCl₃): 7.40-7.18 (10H, m), 7.05 (2H, m), 6.70 (2H, m), 4.57 (1H, m), 3.60 (1H, bd), 3.40 (5H, m), 2.80 (1H, m), 2.60 (1H, m), 2.58 (2H, m), 2.20 (1H, m), 1.90 (1H, m), 1.18 (6H, bs).

¹³C NMR (CDCl₃): 171.4, 149.3, 145.2, 138.9, 129.4, 128.4, 128.1, 128.0, 127.7, 127.2, 126.7, 125.0, 117.1, 60.3, 58.3, 57.8, 53.0, 41 (b), 29.5, 13.4.

An analytical sample was obtained as a hydrochloride by adding an ethereal solution of the free base to ice cold diluted ethereal HCl.

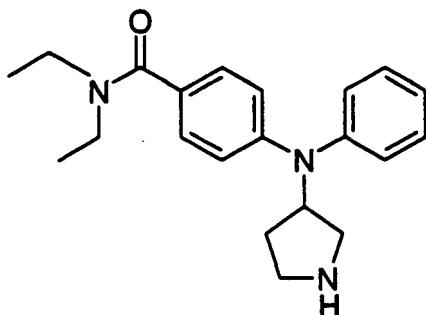
IR (neat): 3430, 1610, 1457 cm⁻¹.

20

Anal. calcd. for C₂₈H₃₃N₃O*HCl*1.3 H₂O: C, 68.99; H, 7.24; N, 8.62.

Found:

C, 68.99; H, 7.57; N, 8.62.

EXAMPLE 16**Preparation of N,N-diethyl-4-(N-pyrrolidin-3-yl-anilino) benzamide (compound 21)**

(21)

5

A mixture of the compound 20 (90 mg, 0.2105 mmol), NH₄O₂CH (27 mg, 0.4282 mmol) and a catalytic amount of 10% Pd/C in MeOH (5 ml) was stirred vigorously at rt overnight. 10 The catalyst was removed through celite and the filtrate condensed in vacuo to give a crude sample which was purified through MPLC (100:0 to 9:1 CH₂Cl₂:MeOH(10%TEA) on silica gel 60) to yield the title compound 21 (30 mg, 42%) as a pale yellow thick oil.

IR (HCl salt, film) v: 3428 (NH), 1607(CONEt₂) cm⁻¹.

15

¹H NMR (free amine, 400 MHz, CDCl₃) δ: 1.06 (6H, m, 2xCH₂CH₃), 1.90 (1H, m, ArNCHCH(CH)CH₂), 2.30 (1H, m, ArNCHCH(CH)CH₂), 2.58 (2H, m, ArNCHCH₂CH₂N), 2.95 (1H, m, NHCH(CH)CH₂), 3.23 (1H, m, NHCH(CH)CH₂), 3.40 (4H, bs, 2xCH₂CH₃), 4.70 (1H, m, ArNCH), 6.68 (2H, m, Ar), 7.02 (2H, m, Ar), 7.22 (3H, m, Ar), 7.38 (2H, m, Ar).

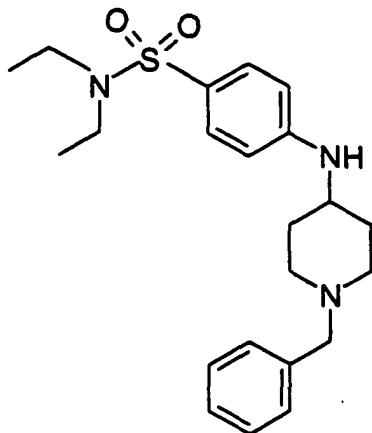
¹³C NMR (free amine, 100Hz, CDCl₃) d: 13.4, 29.7, 41.9, 54.6, 57.9, 59.3, 117.5, 125.5, 127.9, 128.0, 129.7, 144.8, 149.2, 171.2.

Elemental analysis: Calcd. for $C_{21}H_{29}N_3OCl_2 \cdot 1.5H_2O$: C, 57.66; H, 7.37; N, 9.61. Found: C, 57.86; H, 7.38; N, 9.03.

EXAMPLE 17

5

(i) Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzenesulfonamide (compound 22)



(22)

10

Ti(O-i-Pr)₄ (2.10 mL, 7.10 mmol) was added to a mixture of 4-amino-(N,N-diethyl)-benzenesulfonamide (0.81 g, 3.55 mmol) and 1-benzyl-4-piperidone (0.99 mL, 5.32 mmol) at room temperature. The mixture was sonicated at 40 °C for 40 min, and stirred at 60 °C for 18 h. The dark mixture was cooled in an ice-bath and EtOH (15 mL) followed by NaBH₄ pellets (0.5 g, 13.2 mmol) were added. After stirring for 1 h at 0 °C and 20 h at room temperature, 1M NH₄OH (5 mL) was added. The mixture was stirred at room temperature for 30 min, diluted with CH₂Cl₂ (25 mL) and filtered through a pad of Celite®. The layers in the filtrate were separated, the aqueous layer extracted with CH₂Cl₂ (15 mL) and the combined organic phases washed with NaHCO₃ (aq., sat., 25 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) to give the title compound 22 (0.91 g, 46%) as a tan solid.

15

20

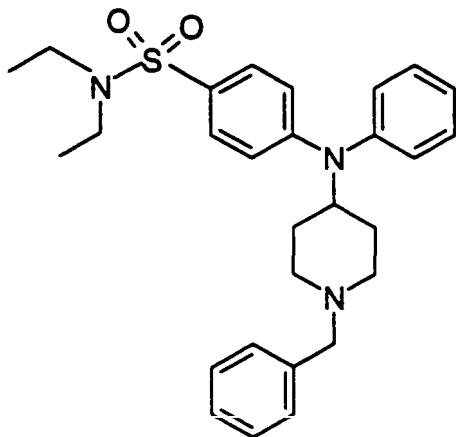
IR (KBr): 2942, 1560, 1520, 1321, 1146, 920 cm^{-1} .

^1H NMR (CDCl_3): 7.55 (d, 2H), 7.34-7.23 (m, 5H), 6.54 (d, 2H), 4.08 (d, 1H), 3.53 (s, 2H), 3.29 (broad s, 1H), 3.17 (q, 4H), 2.85 (d, 2H), 2.16 (t, 2H), 2.01 (d, 2H), 1.51 (q, 2H), 1.11 (t, 6H).

^{13}C NMR (CDCl_3): 150.2, 138.2, 129.1, 129.0, 128.2, 127.0, 126.8, 63.0, 52.1, 49.6, 41.9, 32.2, 14.1.

10

(ii) Preparation of 4-[N-(1-benzyl-piperidin-4-yl)-anilino]-N,N-diethyl benzenesulfonamide (compound 23)



(23)

15

A mixture of 4-[N-(1-benzyl-piperidin-4-yl)-amino]-N,N-diethyl benzenesulfonamide (compound 22), (0.44 g, 1.10 mmol), Ph_3Bi (0.58 g, 1.31 mmol) and $\text{Cu}(\text{OAc})_2$ (0.30 g, 1.64 mmol) in PhMe (20 mL) was heated at reflux for 24 h. Ph_3Bi (0.58 g, 1.31 mmol) and $\text{Cu}(\text{OAc})_2$ (0.30 g, 1.64 mmol) were added. The mixture was stirred at reflux for 24 h and 20 Ph_3Bi (0.58 g, 1.31 mmol) and $\text{Cu}(\text{OAc})_2$ (0.30 g, 1.64 mmol) were added. After reflux for 24 h the mixture was allowed to cool and quenched with 1M NH_4OH (5 mL). The mixture

was stirred at room temperature for 30 min, diluted with EtOAc (25 mL) and filtered through a pad of Celite . The layers in the filtrate were separated, the deep blue aqueous layer extracted with EtOAc (25 mL) and the combined organic phases washed with H₂O (50 mL) and brine (25 mL) and dried over K₂CO₃. The mixture was filtered, concentrated and the residue purified by chromatography (gradient, PhMe to Me₂CO) to give the title compound 23 (50 mg, 10%) as a brown oil.

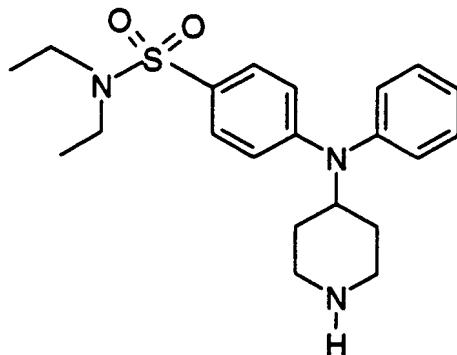
¹H NMR (CDCl₃): 7.51 (d, 2H), 7.43 (t, 2H), 7.35 (t, 1H), 7.30-7.22 (m, 5H), 7.07 (d, 2H), 6.48 (d, 2H), 3.86 (t, 1H), 3.48 (s, 2H), 3.18 (q, 4H), 2.94 (d, 2H), 2.11 (t, 2H), 1.91 (d, 2H), 1.50 (q, 2H), 1.11 (t, 6H).

¹³C NMR (CDCl₃): 151.8, 141.5, 138.1, 131.1, 129.9, 129.1, 128.6, 128.1, 127.5, 127.0, 125.9, 112.7, 63.0, 55.8, 53.1, 42.0, 30.6, 14.2.

Purification with HPLC (LiChroPrep RP-18, elution with increasing amounts of 0.1% TFA/MeCN in 0.1% TFA/H₂O) gave an analytical sample as a white solid.

IR (KBr): 3433, 1677, 1586, 1496, 1324, 1196, 1148, 719 cm⁻¹.

Anal. calcd. for C₂₈H₃₅N₃O₂S*1.25 CF₃COOH: C, 59.07; H, 5.89; N, 6.78.
Found: C, 59.00; H, 6.01; N, 7.01.

EXAMPLE 18**Preparation of N,N-diethyl-4-(N-piperidin-4-yl-anilin) benzenesulfonamide
(compound 24)**

(24)

5

(1-chloroethyl) chloroformate (10 μ L, 0.1 mmol) was added to a solution of 4-[N-(1-benzyl-piperidin-4-yl)-anilino]-N,N-diethyl benzenesulfonamide (**compound 23**) (19 mg, 40 μ mol) in toluene (1 mL) at room temperature. The mixture was heated at reflux for 16h, allowed to cool to room temperature and concentrated. Methanol (1 mL) was added and the mixture was heated at reflux for 4h, allowed to cool and concentrated. The residue was partitioned between CH_2Cl_2 (5 mL) and 1M NH_4OH (5 mL). The layers were separated and the organic phase was washed with H_2O (5 mL) and brine (5 mL) and dried over K_2CO_3 . The mixture was filtered, concentrated and the residue purified by HPLC (LiChroPrep RP-18, elution with increasing amounts of 0.1% TFA/MeCN in 0.1% TFA/ H_2O) to give the title compound **24** (13 mg, 84%) as the trifluoroacetate.

IR (KBr): 3420, 1658, 1199, 1146, 714 cm^{-1} .

20 ^1H NMR (CD_3OD : δ : 7.66-7.63 (m, 4H), 7.55 (t, 1H), 7.28 (d, 2H), 6.76 (d, 2H), 4.50 (t, 1H), 3.54 (d, 2H), 3.35-3.23 (m, 6H), 2.34 (d, 2H), 1.73 (q, 2H), 1.19 (t, 6H).

¹³C NMR (CD₃OD) δ: 153.4, 142.4, 132.8, 131.7, 130.1, 129.7, 128.8, 114.4, 53.6, 45.2, 43.6, 29.2, 14.9.

Anal. calcd. for $C_{24}H_{33}N_3O_2S \times 2 CF_3COOH \times 1.5 H_2O$: C, 46.73; H, 5.33; N, 6.54

5 Found: C, 46.54; H, 5.01; N, 6.71

The best mode of performing the invention known at present, is to use the compounds of
10 Example 1, 2, 3, 4, 5, 6, 7, 17 and 18.

Pharmaceutical compositions

15 The novel compounds according to the present invention may be administered orally, intramuscularly, subcutaneously, topically, intranasally, intraperitoneally, intrathoracically, intravenously, epidurally, intrathecally, intracerebroventricularly and by injection into the joints.

20 A preferred route of administration is orally, intravenously or intramuscularly.

The dosage will depend on the route of administration, the severity of the disease, age and weight of the patient and other factors normally considered by the attending physician, when determining the individual regimen and dosage level at the most appropriate for a particular patient.

For preparing pharmaceutical compositions from the compounds of this invention, inert, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, and suppositories.

A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material.

- 5 In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component. In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.
- 10 For preparing suppository compositions, a low-melting wax such as a mixture of fatty acid glycerides and cocoa butter is first melted and the active ingredient is dispersed therein by, for example, stirring. The molten homogeneous mixture is then poured into convenient sized molds and allowed to cool and solidify.
- 15 Suitable carriers are magnesium carbonate, magnesium stearate, talc, lactose, sugar, pectin, dextrin, starch, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low-melting wax, cocoa butter, and the like.

Pharmaceutically acceptable salts are acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium acetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glucaptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrabamine, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate mesylate, methylbromide, methylnitrate, methylsulfate, mucate, napsylate, nitrate, pamoate (embonate), pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, triethiodide, benzathine, chloroprocaine, choline, diethanolamine, ethylenediamine, meglumine, procaine, aluminium, calcium, lithium, magnesium, potassium, sodium, and zinc.

Preferred pharmaceutically acceptable salts are the hydrochlorides, trifluoroacetates and bitartrates.

The term composition is intended to include the formulation of the active component with
5 encapsulating material as a carrier providing a capsule in which the active component (with or without other carriers) is surrounded by a carrier which is thus in association with it.
Similarly, cachets are included.

Tablets, powders, cachets, and capsules can be used as solid dosage forms suitable for oral
10 administration.

Liquid from compositions include solutions, suspensions, and emulsions. Sterile water or
water-propylene glycol solutions of the active compounds may be mentioned as an
example of liquid preparations suitable for parenteral administration. Liquid compositions
15 can also be formulated in solution in aqueous polyethylene glycol solution.

Aqueous solutions for oral administration can be prepared by dissolving the active
component in water and adding suitable colorants, flavoring agents, stabilizers, and
thickening agents as desired. Aqueous suspensions for oral use can be made by dispersing
20 the finely divided active component in water together with a viscous material such as
natural synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other
suspending agents known to the pharmaceutical formulation art.

Preferably the pharmaceutical compositions is in unit dosage form. In such form, the
25 composition is divided into unit doses containing appropriate quantities of the active
component. The unit dosage form can be a packaged preparation, the package containing
discrete quantities of the preparations, for example, packeted tablets, capsules, and powders
in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself, or
it can be the appropriate number of any of these packaged forms.

BIOLOGICAL EVALUATION

A) IN VITRO MODEL

Cell culture

5

Human 293S cells expressing cloned human μ , δ , and κ receptors and neomycin resistance were grown in suspension at 37°C and 5% CO₂ in shaker flasks containing calcium-free DMEM 10% FBS, 5% BCS, 0.1% Pluronic F-68, and 600 μ g/ml geneticin.

10

Membrane preparation

15

Cells were pelleted and resuspended in lysis buffer (50 mM Tris, pH 7.0, 2.5 mM EDTA, with PMSF added just prior to use to 0.1 mM from a 0.1 M stock in ethanol), incubated on ice for 15 min, then homogenized with a polytron for 30 sec. The suspension was spun at 1000g (max) for 10 min at 4°C. The supernatant was saved on ice and the pellets resuspended and spun as before. The supernatants from both spins were combined and spun at 46,000 g(max) for 30 min. The pellets were resuspended in cold Tris buffer (50 mM Tris/Cl, pH 7.0) and spun again. The final pellets were resuspended in membrane buffer (50 mM Tris, 0.32 M sucrose, pH 7.0). Aliquots (1 ml) in polypropylene tubes were frozen in dry ice/ethanol and stored at -70°C until use. The protein concentrations were determined by a modified Lowry assay with SDS.

Binding assays

25

Membranes were thawed at 37°C, cooled on ice, passed 3 times through a 25-gauge needle, and diluted into binding buffer (50 mM Tris, 3 mM MgCl₂, 1 mg/ml BSA (Sigma A-7888), pH 7.4, which was stored at 4°C after filtration through a 0.22 m filter, and to which had been freshly added 5 μ g/ml aprotinin, 10 μ M bestatin, 10 μ M diprotin A, no DTT). Aliquots of 100 μ l (for μ g protein, see Table 1) were added to iced 12x75 mm polypropylene tubes containing 100 μ l of the appropriate radioligand (see Table 1) and

100 μ l of test peptides at various concentrations. Total (TB) and nonspecific (NS) binding were determined in the absence and presence of 10 μ M naloxone respectively. The tubes were vortexed and incubated at 25°C for 60-75 min, after which time the contents are rapidly vacuum-filtered and washed with about 12 ml/tube iced wash buffer (50 mM Tris, 5 pH 7.0, 3 mM MgCl₂) through GF/B filters (Whatman) presoaked for at least 2 h in 0.1% polyethyleneimine. The radioactivity (dpm) retained on the filters was measured with a beta counter after soaking the filters for at least 12 h in mini vials containing 6-7 ml scintillation fluid. If the assay is set up in 96-place deep well plates, the filtration is over 96-place PEI-soaked unifilters, which were washed with 3 x 1 ml wash buffer, and dried in 10 an oven at 55°C for 2 h. The filter plates were counted in a TopCount (Packard) after adding 50 μ l MS-20 scintillation fluid/well.

Data analysis

15 The specific binding (SB) was calculated as TB-NS, and the SB in the presence of various test peptides was expressed as percentage of control SB. Values of IC₅₀ and Hill coefficient (n_H) for ligands in displacing specifically bound radioligand were calculated from logit plots or curve fitting programs such as Ligand, GraphPad Prism, SigmaPlot, or ReceptorFit. Values of K_i were calculated from the Cheng-Prusoff equation. Mean \pm 20 S.E.M. values of IC₅₀, K_i and n_H were reported for ligands tested in at least three displacement curves.

Receptor saturation experiments

Radioligand K_s values were determined by performing the binding assays on cell membranes with the appropriate radioligands at concentrations ranging from 0.2 to 5 times the estimated K_s (up to 10 times if amounts of radioligand required are feasible). The specific radioligand binding was expressed as pmole/mg membrane protein. Values of K_s and B_{max} from individual experiments were obtained from nonlinear fits of specifically bound (B) vs. nM free (F) radioligand from individual according to a one-site model.

10 B) BIOLOGICAL MODEL (IN VIVO MODEL)

FREUND'S COMPLETE ADJUVANT (FCA), AND SCIATIC NERVE CUFF
INDUCED MECHANO-ALLODYNIA IN RAT

Animals

15 Male Sprague-Dawley rats (Charles River, St-Constant, Canada) weighing 175-200g at the time of surgery were used. They were housed in groups of three in rooms thermostatically maintained at 20° C with a 12:12 hr light/dark cycle, and with free access to food and water. After arrival, the animals were allowed to acclimatize for at least 2 days before surgery. The experiments were approved by the appropriate Medical Ethical Committee
20 for animal studies.

EXPERIMENTAL PROCEDUREFREUND'S COMPLETE ADJUVANT

The rats were first anesthetized in a Halothane chamber after which 10 μ l of FCA was
25 injected s.c. into the dorsal region of the left foot, between the second and third external digits. The animals were then allowed to recover from anesthesia under observation in their home cage.

SCIATIC NERVE CUFF

The animals were prepared according to the method described by Mosconi and Kruger (1996). Rats were anesthetized with a mixture of Ketamine / Xylazine i.p. (2ml/kg) and placed on their right side and an incision made over, and along the axis of, the lateral aspect of the left femur. The muscles of the upper quadriceps were teased apart to reveal the sciatic nerve on which a plastic cuff (PE-60 tubing, 2mm long) was placed around. The wound was then closed in two layers with 3-0 vicryl and silk sutures.

DETERMINATION OF MECHANO-ALLODYNIA USING VON FREY TESTING

Testing was performed between 08:00 and 16:00h using the method described by Chapman et al. (1994). Rats were placed in Plexiglas cages on top of a wire mesh bottom which allowed access to the paw, and were left to habituate for 10-15 min. The area tested was the mid-plantar left hind paw, avoiding the less sensitive foot pads. The paw was touched with a series of 8 Von Frey hairs with logarithmically incremental stiffness (0.41, 0.69, 1.20, 2.04, 3.63, 5.50, 8.51, and 15.14 grams; Stoelting, Ill, USA). The von Frey hair was applied from underneath the mesh floor perpendicular to the plantar surface with sufficient force to cause a slight buckling against the paw, and held for approximately 6-8 seconds. A positive response was noted if the paw was sharply withdrawn. Flinching immediately upon removal of the hair was also considered a positive response. Ambulation was considered an ambiguous response, and in such cases the stimulus was repeated.

TESTING PROTOCOL

The animals were tested on postoperative day 1 for the FCA-treated group and on post-operative day 7 for the Sciatic Nerve Cuff group. The 50% withdrawal threshold was determined using the up-down method of Dixon (1980). Testing was started with the 2.04 g hair, in the middle of the series. Stimuli were always presented in a consecutive way, whether ascending or descending. In the absence of a paw withdrawal response to the initially selected hair, a stronger stimulus was presented; in the event of paw withdrawal, the next weaker stimulus was chosen. Optimal threshold calculation by this method

requires 6 responses in the immediate vicinity of the 50% threshold, and counting of these 6 responses began when the first change in response occurred, e.g. the threshold was first crossed. In cases where thresholds fell outside the range of stimuli, values of 15.14 (normal sensitivity) or 0.41 (maximally allodynic) were respectively assigned. The 5 resulting pattern of positive and negative responses was tabulated using the convention, X = no withdrawal; O = withdrawal, and the 50% withdrawal threshold was interpolated using the formula:

$$50\% \text{ g threshold} = 10^{(X_f + k\delta)} / 10,000$$

10

where X_f = value of the last von Frey hair used (log units); k = tabular value (from Chaplan et al. (1994)) for the pattern of positive / negative responses; and δ = mean difference between stimuli (log units). Here δ = 0.224.

15 Von Frey thresholds were converted to percent of maximum possible effect (% MPE), according to Chaplan et al. 1994. The following equation was used to compute % MPE:

$$\% \text{ MPE} = \frac{\text{Drug treated threshold (g)} - \text{allodynia threshold (g)}}{\text{Control threshold (g)} - \text{allodynia threshold (g)}} \times 100$$

20

ADMINISTRATION OF TEST SUBSTANCE

Rats were injected (subcutaneously, intraperitoneally, or orally) with a test substance prior to von Frey testing, the time between administration of test compound and the von Frey test 25 varied depending upon the nature of the test compound.